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(a) isolating, chemically synthesizing or amplifying with polymerase chain reaction (PCR) a cDNA, mRNA or genomic DNA encoding a light or heavy chain of the antigen-specific antibodies and assembling the antibodies cDNA encoding said light and heavy chains of said antibodies into two separate expression cassettes, one encoding DNA for the light chain and the second encoding DNA for the heavy chain, each cassette further comprising a flanking signal DNA sequence preceded by a yeast promoter at 5' terminus and by the yeast transcription termination DNA sequence of the 3'-terminus;

(b) preparing a recombinant *Pichia pastoris* (*P. pastoris*) yeast expression vector pPICZ $\alpha$  by restriction digestion with EcoRI and BamHI;

(c) constructing a recombinant *P. pastoris* yeast expression plasmid containing the expression cassettes of step (a);

(d) cloning the expression cassettes of step (c) into the *P. pastoris* expression vector pPICZ $\alpha$  to generate recombinant plasmid pPICZ $\alpha$ LH comprising expression cassettes for the light and heavy chains;

(e) transforming *Saccharomyces cerevisiae* with the recombinant plasmid by placing said expression cassettes of step (d) under the control of the AOX1 promoter fused to the DNA encoding the *Saccharomyces cerevisiae*  $\alpha$ -mating factor signal;

(f) amplifying and isolating the recombinant plasmid;

(g) transforming *P. pastoris* spheroblasts with *Bgl*II

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linearized, *NotI* linearized, *SacI* linearized, *SalI* linearized or *StuI*-linearized recombinant plasmid replacing the yeast chromosomal *AOX1* DNA sequence with *AOX1*-antibody DNA sequence containing expression cassettes of the recombinant plasmid of step (d);

(h) selectively growing the recombinants;

(i) screening yeast transformation colonies for a recombinant antibody expression;

(j) analyzing putative positive yeast clones for chromosomal integrates of the expression cassettes of heavy and light chain cDNAs;

(k) confirming the integrity of the DNA insert;

(l) inducing the recombinant antibody expression;

(m) confirming the intactness of the expression cassettes inserts with PCR and Northern blot analysis;

(n) detecting the presence of the recombinant antibody by Western blot;

(o) testing the recombinant antibody for specific antigen-antibody binding, and

(p) harvesting the antigen-specific antibody produced in steps (a) - (o),

wherein said antibody is produced in quantity of 10-36 mg/l in about 12 to about 108 hours.

23. (New) The method of claim 22 wherein the antibody cDNA is assembled into the expression cassettes by subcloning the antibody light and heavy chain cDNA in tandem as *EcoRI-BglIII/BsmBI* fragments

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flanked by a DNA encoding the *P. pastoris* signal sequence, preceded by a *P. pastoris* promoter at the 5'-terminus and by a *P. pastoris* yeast transcription termination DNA sequence at the 3'-terminus.

24. (New) The method of claim 23 wherein the signal sequence is a yeast  $\alpha$ -factor and wherein the promoter is an alcohol oxidase AOX1-P.

*SUB 52* 25. (New) The method of claim 24 wherein the antigen is dioxin.

*F1* 26. (New) The method of claim 25 wherein the antibody cDNA encoding the light and heavy chain is isolated from a hybridoma DDI (ATCC Accession Number HB9741) that recognizes dioxin.

*SUB 63* 27. (New) The method of claim 24 wherein the replacement of the yeast chromosomal AOX1 with AOX1-antibody cDNA containing cassettes is by homologous recombination replacement.

28. (New) The method of claim 27 wherein the selective growth of the recombinants and elimination of non-recombinants is performed on a medium containing zeocin.

29. (New) The method of claim 27 wherein the selective growth of the recombinants is performed on a medium containing g418, trimethoprin, or a compound that limits the growth of wild type *P.*

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30. (New) The method of claim 28 wherein the screening of transformed colonies for antibody expression is by colony-immunoblotting.

31. (New) The method of claim 30 wherein the analysis of putative positive clones of step (j) is by a PCR or by a restriction analysis.

32. (New) The method of claim 31 wherein the integrity of the cDNA inserts is confirmed by nucleotide sequence analysis.

*E/  
w*

33. (New) A recombinant *Pichia pastoris* (*P. pastoris*) yeast expression vector containing dual expression cassettes, each cassette carrying an entire cDNA copy of immunoglobulin light or heavy chain DNA and further comprising a flanking signal DNA sequence preceded by a yeast promoter at 5'-terminus and by the yeast termination DNA sequence of the 3'-terminus, said vector useful in a method for production of a recombinant antigen-specific antibody in amounts from about 10 to about 36 mg/l in about 12 to about 108 hours.

34. (New) An expression vector comprising *Pichia pastoris* (*P. pastoris*) transformed with human, mouse or humanized mouse immunoglobulin monoclonal cDNA for production of an entire

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recombinant antigen-specific intact antibody in amounts from about 10 to about 36 mg/l in about 12 to about 108 hours.

*PL*

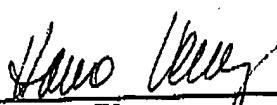
35. (New) *Pichia pastoris* (*P. pastoris*) yeast transformed with two expression cassettes one of which carries a cDNA of a light chain of an anti-dioxin immunoglobulin and the second of which carries a cDNA of a heavy chain of an anti-dioxin immunoglobulin, said transformed yeast useful in the method for production of intact antibodies isolated from DD1 hybridoma (ATCC Accession Number HB9741), in amounts from about 10 to about 36 mg/l in from about 12 to about 108 hours.

REMARKS

This Preliminary Amendment is filed following Examiner's request to resubmit the Preliminary Amendment for examination of the Continuing Prosecution Application. Examiner is respectfully requested to examine claims 22-35.

Respectfully submitted,

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